DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: NOV 13 2003

4534 '04 JAN -5 P1:58

From: Pharmacologist, Division of Dietary Supplement Programs and Compliance, Office of Nutritional Products, Labeling, and Dietary Supplements, HFS-810

Subject: 75-Day Premarket Notification of New Dietary Ingredients

To: Dockets Management Branch, HFA-305

Subject of the Notification:	Ganoderma Lucidum Spore Powder [Lingzhi Master Brand]
Firm:	Care & Health Limited
Date Received by FDA:	February 5, 2003
90-Day Date:	May 5, 2003

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned substance should be placed on public display in docket number

Thank you for your assistance.

Susan C. Aitken

CSO/Lead Reviewer
Susan C. Aitken, Ph.D.

Attachments

955-0316

RPT173

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD

APR 21 2003

Ms. Anita Lam
Assistant Marketing Manager
Care & Health Limited Unit 1, 4/F, Block B
Shatin Industrial Centre
5-7 Yen Shun Circuit
Shatin, N.T., Hong Kong

Dear Ms. Lam:

This letter is to inform you that the Food and Drug Administration (FDA) received your notification of intent to market Ganoderma lucidum spore powder as a new dietary ingredient pursuant to 21 U.S.C. 350b(a)(2) on January 28, 2003. The notification indicated the product would be marketed under the brand name Lingzhi Master. One original and two copies were needed to proceed with the notification under Title 21 of the Code of Federal Regulations (21 CFR) Part 190.6. As you originally submitted a single copy, you then submitted two additional copies of the notification. These copies were received on February 5, 2003 and, therefore, the filing date will be recorded as February 5, 2003.

In accordance with 21 C.F.R 190.6(c), FDA must acknowledge its receipt of a notification for a new dietary ingredient. For 75 days after the filing date (i.e. until April 21, 2003), your client must not introduce or deliver for introduction into interstate commerce any dietary supplement that contains Ganoderma lucidum spore powder.

Please note that acceptance of this notification for filing is a procedural matter and, thus, does not constitute a finding by FDA that the new dietary ingredient or supplement that contains the new dietary ingredient is safe or is not adulterated under 21 U.S.C. 342. FDA is not precluded from taking action in the future against any dietary supplement containing Ganoderma lucidum spore powder if it is found to be unsafe, adulterated, or misbranded. As another procedural matter, your notification will be kept confidential for 90 days after the filing date. After May 6, 2003, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. Although any confidential or proprietary business information in the notification will not be discussed with the public, you may wish to indicate in writing what exact information you believe may be proprietary.

CARE & HEALTH LIMITED
寫字樓：香港新界沙田源順圍5-7號沙田工業中心B座4樓1室
康而健會：香港九龍尖沙咀赫德道8號25樓E至F室
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康而健有限公司

Division of Standard and Labeling Regulations
Office of Nutrition Products, Labeling and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740 – 3835
USA

21 January 2003

To Whom It May Concern,

Re: Application for the New Dietary Ingredient in USA

As we are going to market a dietary supplements that contains new dietary ingredient, I am writing to notify about this ingredient.

The new dietary ingredient that we are going to market is *Ganoderma lucidum* Spore which is the spore of *Ganoderma lucidum*, a precious herb traditional Chinese medicine. The product is in capsule form and the brand name is Lingzhi Master.

Enclose please find the detail product information including the herb name, manufacturing process and the safety of the product for your consideration. I would be grateful if you can consider our product as the new dietary ingredient and the permission for market this product in USA.

Please do not hesitate to contact me if you have further question by mail. Moreover, you can also reach me at email: anitalam@hanison.com.hk

Best regards,

Anita

Anita Lam
Assistant Marketing Manager

RECEIVED
1/28/03 BJS

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康而健有限公司

Division of Standards and Labeling Regulations
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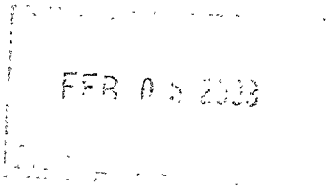


29 January 2003

To whom it may concern,

Re: Notification of New Dietary Ingredient in FDA

My name is Anita Lam from Care & Health Ltd. in Hong Kong. I am going to submit additional copies for the notification of new dietary ingredient.

I have already submitted one copy of information about the *Ganoderma lucidum* Spore Powder (brand name: Lingzhi Master) for the notification. However, today I received an email from FDA because total three copies is required. Therefore, I am going to send you two more copies. Please kindly receive the enclosed copies so that the notification can be proceed. Thank you very much.

Please do not hesitate to contact me if you need further information. My email address is anitalam@hansion.com.hk.

Best regards,

Anita

Anita Lam
Assistant Marketing Manager

Application of *Ganoderma lucidum* Spore Powder as New Dietary Ingredient in USA

Company: Care & Health Ltd
Address: Unit 1, 4/F, Block B, Shatin Industrial Center, 5-7, Yuen Shun Circuit. Shatin, N.T., Hong Kong
Date: 21st January 2003

Content

Introduction

Lingzhi Master Ganoderma Spore Powder

1. Introduction

1.1 Ganoderma lucidum – traditional Chinese medicine

Ganoderma lucidum is a basidiomydetes. The family of basidiomycetes consists of over 25,000 different species of fungi including mushroom. *Ganoderma lucidum* is also referred as "Lingzhi" in China or Reishi in Japan. It is considered a medicinal mushroom and a precious herb in traditional Chinese medicine. The entire mushroom is used. *Ganoderma lucidum* is recorded in the Chinese medical classic. It is described in details in Compendium of Material Medica written by Li Shi Zhen. He is a famous medical scholar in Ming Dynasty (1368 – 1644). As Lingzhi was also prominently mentioned in the famous 14th century Chinese folklore " The story of a White Snake", it has been widely known in the Orient and considered a very valuable herb.

The use of *Ganoderma lucidum* described in ancient Chinese medical books is to promote longevity and maintain vitality of the human body. Many beneficial effects of *Ganoderma lucidum* have been claimed. Most of these claims have not been studied in controlled clinical trials, although there has been an abundance of clinical use, in vitro and animal data. The major benefit of the herb appears to be its immuno-modulating action, resulting in enhancement of immunity, improvement of liver functions, improvement and restoration of the normal function of the respiratory system, and prevention of certain viral infections. The anti-hypertensive action fo the herb demonstrated in the animal may be important in regulating the cardiovascular system and lowering blood pressure in humans. The anti-tumor and anti-HIV protease activities found in vitro and in the animal may help explain the claims that G. lucidum could prevent cancer, hepatitis B, hepatitis C and hepatoma, although clinical data to support the claim is not available.

1.2 Ganoderma lucidum Spore

A mature *Ganoderma lucidum* mushroom produces million of spore. The spores are ejected into the air when mature and forming a light cloud near the mother mushroom.

Therefore, the spores also called spore powder. Under and electron microscope, the spore is oval shaped which is about 8-12 X 6-7 μm. Each spore covered with two layers of tough wall which is called sporoderm. Natural germination process is very slow with a low germination rate. Even under optimal condition, budding occurs in 24 to 48 hours, an mycelia formed after 72 hours with a germination rate of 3-15%

1.3 The New Dietary Ingredient: Ganoderma lucidum Spore Powder

Each *Ganoderma lucidum* spore consists of full copy of gene for the plant. The bio-ingredient is synthesized during the germination process. Ganoderma spore are also used as herb. However, as the spore is covered by two layers of tough wall, it is questionable whether the nutrient inside the spores can be effectively absorbed.

The new dietary ingredient, *Ganoderma lucidum* Spore Powder, is made from process spores. Patented procedure is used which is first to select the spores, promote germination and micro-break the sporoderm. The purpose of this process is to promote the synthesis of bio-ingredient, disrupt the sporoderm to enhance body absorption, and retain the activities of the bio-ingredient throughout the processing procedures. After further drying and refinement, the powder formed is the dietary ingredient, *Ganoderma lucidum* Spore Powder. Then the powder encapsuled to produce the branded product, Lingzhi Master Ganoderma Spore Powder.

The human uses of the mushroom of *Ganoderma lucidum* have been documented for a long period of time. In comparison, the use of pure *Ganoderma lucidum* spore is of recent decades. The new dietary ingredient has been subjected to toxicological studies to ensure its safety.

Product Specification

2. *Product Specification*

2.1 Product Specification

Product Name:	Lingzhi Master Ganoderma Spore Capsule
Ingredient:	Each gelatin capsule contains 300mg of *Ganoderma lucidum* Spore Powder (100%)
Botanical Name:	*Ganoderma lucidum* Spore
Distributor:	Care & Health Limited
Distributor Address:	Unit 1, 4/F, Block B, Shatin Industrial Center, 5-7, Yuen Shun Circuit, Shatin, N.T., Hong Kong

Heavy Metal:		
	Arsenic	< 1.0ppm
	Cadmium	< 1.0ppm
	Mercury	< 0.5ppm
	Lead	< 1.0ppm
	Copper	< 20ppm

Microorganism:		
	Total plate count	Less than 10 CFU/g
	E. coli	Less than 30CFU/100g
	Pathogenic Bacterial	Not detected

Packaging:	300mg/capsule 60 or 150 capsule / bottle Net weight is 18g for 60's and 45g for 150's
Dosage:	4 capsules to be taken 3-4 times daily.

2.2 Testing Report

Test report date: 29-1-2002

The original report in Chinese is attached in Appendix A

Category	Test Item	Unit	Result		
			Batch Number		
			010910	010925	011012
Heavy metal	Lead	mg/kg	0.34	0.31	0.31
	Aresenic	mg/kg	< 0.1	< 0.1	< 0.1
	Mercury	mg/kg	0.020	0.019	0.022
Pesticide	Hexachlorocyclohexane	mg/kg	< 0.01	< 0.01	< 0.01
	DDT	mg/kg	< 0.01	< 0.01	< 0.01
Pathogen Screen	Total plate count	cfu/g	< 10	< 10	< 10
	E.coli	MPN/100g	< 30	< 30	< 30
	Mold	cfu/g	10	10	< 10
	Yeast	cfu/g	< 10	< 10	< 10
	Salmonella		Not detected		
	Staphylococcus		Not detected		
Nutrition	Water	g/100g	2.45	2.40	2.41
	Polyssaccharide	g/100g	3.12	3.23	3.07
	Unsaturated fatty acid	%	25.05	25.54	28.04

Manufacturing Process

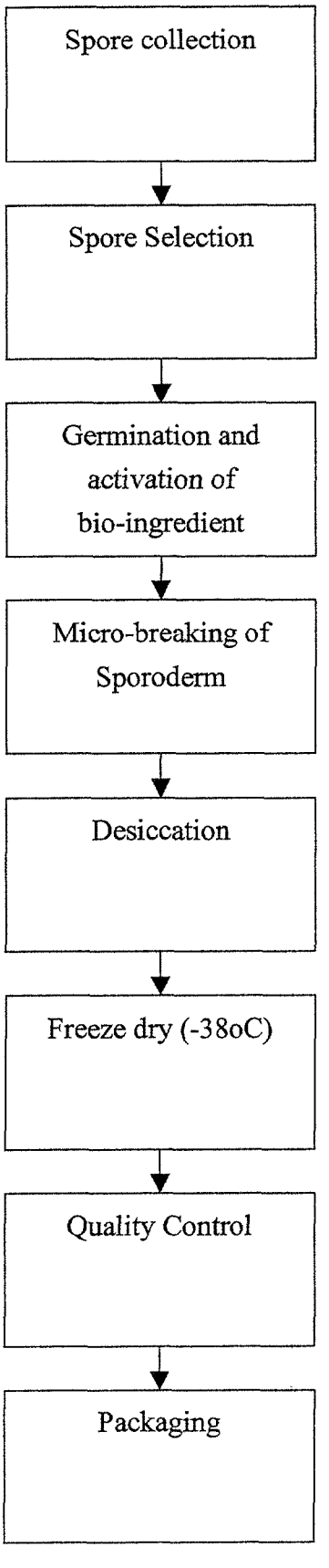
3. Manufacturing Process

A flow chat of the manufacturing process is presented in the following page

The new dietary ingredient, Lingzhi Master *Ganoderma lucidum* Spore Powder, is manufactured using a patented technique, which can be described as sporo-germination activation and microbreaking of sporoderm

The process is briefly described as follow; the spores are collected from the fungi, *Ganoderma lucidum*. Then, matured well-formed spores are selected. The selected spores then undergo sporo-germination activation, which is cultivation in liquid media under controlled temperature for a certain period of time, followed with incubation under controlled temperature and humidity. Successful germination rate is 95% or higher. The spores are further subjected to enzyme treatment at low temperature to micro-break the sporoderm while preserves the bio-ingredients in their active state. The processed spores and then dried through desiccation and freeze-drying, and finally refined into powder. The quality of Lingzhi Master *Ganoderma lucidum* Spore Powder is then encapsulated in gelatin capsules and packed to product the final product, Lingzhi Master Ganoderma Spore Powder.

Manufacturing Process Flow Chart



Toxicity Studies

4. *Summary of safety for Lingzhi Master Ganoderma Spore Powder*

Five toxicity studies of the dietary ingredient, *Ganoderma lucidum* Spore Powder (brand name: Lingzhi Master Ganoderma Spore Powder) were performed in 1999 in Food Safety and Inspection Laboratory, Institute of Health, Guang Dong Province. The original copy (in Chinese) of the report is attached in Appendix B. The result are summarized as follow:

1. *Acute toxicity*

   Acute toxicity test in mice shown that the LD_{50} of *Ganoderma lucidum* Spore Powder was more than 21.5g/kg body weight. The highest dose used in the study is more than 800 times higher than the recommended maximum dose of 1.8g for an average 70kg human

2. *Accumulative toxicity study*

   The accumulative toxicity study in mice, receiving an accumulative dose of 2.5g, 5g and 10g respectively per 100g of food, demonstrate that np significant effect on body weight of the test animals. No abnormalities were observed int eh internal organs examined compared to control groups.

3. *Mutagenesis*

   Ganoderma lucidum Spore Powder at 1.62 – 10g / kg b.w. did not induce mutation of the female bone marrow cells in the mouse bone marrow micronucleus test.

4. *Malformation of mouse sperm*

   Ganoderma lucidum Spore Powder at 2.5 – 10g / kg b.w. did not induce abnormalities in mouse sperm

5. *Ames test*

   The Ames test indicated that *Ganoderma lucidum* Spore Powder did not induce directly or indirectly mutation in the bacterial strains tested.

6. *Conclusion*

It is therefore concluded that *Ganoderma lucidum* Spore Powder was not toxic to the test animals at the doses used. The results of the animal toxicity tests are presented in the following section. Base upon the toxicity study results, we conclude that Ganoderma Spore Powder is considered safe to use at the recommended maximum oral daily dose of 4.8g (**300mg / capsule**, 4 capsules to be taken 3-4 times daily)

5. *Toxicity in Animals*

This section presents the result of the five toxicity studies reported in the Chinese document for *Ganoderma lucidum* Spore Powder, which is summarized in section 4. The original Chinese document is attached in Appendix B.

5.1 Acute Toxicity

5.1.1 Testing Institute

- Food Safety and Inspection Laboratory, Institute of Health, Guang Dong Province, China.

5.1.2 Testing Date

- 16th November, 1999

5.1.3 Material

- Testing compound: 120g *Ganoderma lucidum* Spore Powder was used, sieved and mixed with 300ml of distilled water.
- Animal: Healthy, white NIH mice supplied by Guang Dong Province Medical Animal Farm, China. The weight of mice between 18-22g

5.1.4 Methods

- 40 NIH mice (20 females and 20 males), weight between 18 to 22g,were randomized into four dose groups according to the Horn's methodology. One dose of the test product was given to the mice by gavage on empty stomach.

5.1.5 Result

- The mice were observed for 1 week. The result are shown in table 5.1

Dose (g / kg)	No. of Animals		No. of Dead Animals	
	Male	Female	Male	Female
21.50	5	5	0	0
10.00	5	5	0	0
4.64	5	5	0	0
2.15	5	5	0	0

Table 5.1 Result of acute toxicity of *Ganoderma lucidum* Spore Powder

5.1.6 Conclusion

- No adverse reaction to the test compound was observed in mice. The LD50 value was therefore larger than 21.5g/kg body weight. The test compound was determined to be non-toxic.

5.2 Accumulative Toxicity

5.2.1 Testing Institute

- Food Safety and Inspection Laboratory, Institute of Health, Guang Dong Province, China.

5.2.2 Testing Date

- 15th December, 1999

5.2.3 Material

- Testing compound: Feed mixed with 2.5g, 5g and 10g of *Ganoderma lucidum* Spore Powder respectively.

- Animals: SD mice supplied by Medical Animal Farm in Guang Dong Province.

5.2.4 Methods

- 96 SD mice with weight between 80-88g were used. The mice were divided into 4 groups with 24 mice in each group. The mice were fed by feed, 100g of feed with 2.5g, with 5.0g and with 10.0g of *Ganoderma lucidum* Spore Powder respectively for 30 days. The classification of dosage group is

    a) Control: No *Ganoderma lucidum* Spore Powder was added

    b) Low Dose: 100g feed mix with 2.5g of Spore Powder

    c) Medium Dose: 100g feed mix with 5g of Spore Powder

    d) High Dose: 100g feed mix with 10g of Spore Powder

  Measure the body weight and the consumption of feed once a week.

- Measure the RBC count, WBC count, hemoglobin and platelet count at the end of the study.

- Measure the biochemical index such as blood glucose, triacylglycerol, total cholesterol and protein.

- Observe the liver, kidney, spleen heart and testis by biopsy.

5.2.5 Result

- Body weight: There were no significant difference in body weight between all treatment groups compare with control group. ($P>0.05$)

- Blood Test: WBC in male increase, $F=5.14$, for the low and medium dose group, it shown the significant different. For other test, no significant different were found.

- Blood biochemical test: Blood glucose female medium and high dose group increase, $F=3.93$ with significant different ($P<0.05$). Nitrogen in male medium and low dose group decrease, $F=4.49$ with significant different but all the fluctuation are within the normal range. For other indicators shown non-significant different. ($P>0.05$)

- Observation of organ: no abnormalities were observed.

5.2.6 Conclusion

- The *Ganoderma lucidum* Spore Powder did not have any accumulative toxicity in mice

5.3 Mouse Bone Marrow Micronucleus Test

5.3.1 Testing Institute

- Food Safety and Inspection Laboratory, Institute of Health, Guang Dong Province, China.

5.3.2 Testing Date

- 16th November, 1999

5.3.3 Material

- Testing compound: 120g *Ganoderma lucidum* Spore Powder was used, sieved and mixed with 300ml of distilled water.

- Animal: Healthy, white NIH mice supplied by Guang Dong Province Medical Animal Farm, China. The weight of mice between 20-23g

5.3.4 Methods

- 70 NIH mice, weight between 20 to 23g, were placed into 7 groups of 0, 0.062, 1.25, 2.50, 5.00, 10.00 g/kg and 0.06 g/kg of CTX. Each group consisted of 10 mice, 5 females and 5 males. The seventh group was the positive control group (cyclophosphamide). The experiment was performed according to the "Procedure of Toxicity Evaluation for Food Safety". The mice were dosed in two gavage administrations. The mice were sacrificed six hours after the second administration. The bone marrow from both femurs was removed and made into slides, which were subsequently stained and examined under microscope. The result is shown in 5.2

5.3.5 Result

Table 5.2 Result of mouse micronucleus test using *Ganoderma lucidum* Spore Powder

Dose (g/kg)	**No. of Animals**		**No. of PCE examined**		**No. of MN-PCE**		**Micronucleated Ratio (‰)**	
	Male	Female	Male	Female	Male	Female	Male	Female
0	5	5	5000	5000	7	7	1.4	1.4
10.00	5	5	5000	5000	8	7	1.6	1.4
5.00	5	5	5000	5000	6	8	1.2	1.6
2.50	5	5	5000	5000	6	7	1.2	1.4
1.25	5	5	5000	5000	7	7	1.4	1.4
0.62	5	5	5000	5000	6	6	1.2	1.2
CTX (0.06)	5	5	5000	5000	123	126	24.6**	25.2**

** The placebo control group and the treatment group were compared to the cyclophosphamide positive control, $p<0.001$,

5.3.6 Conclusion

- No significant differences among the Micronucleated Ratio of the placebo control and the dose groups were observed. In comparison, the cyclophosphamide positive control group was significantly different from the placebo control group ($P<0.001$). The experimental result indicated that the *Ganoderma lucidum* Spore Powder did not induce mutation in the mouse micronucleus test.

5.4 Study in Malformation of mouse sperms

5.4.1 Testing Institute

- Food Safety and Inspection Laboratory, Institute of Health, Guang Dong Province, China.

5.4.2 Testing Date

- 16th November, 1999

5.4.3 Material

- Testing compound: 120g *Ganoderma lucidum* Spore Powder was used, sieved and mixed with 300ml of distilled water.

- Animal: Healthy, white NIH mice supplied by Guang Dong Province Medical Animal Farm, China. The weight of mice between 18-22g

5.4.4 Methods

- 25 NIH male mice, weight between 18-22g, were randomized into 5 groups. They were given the test compound once a day by gavage for 5 consecutive days, at the dose levels of 0, 2.50, 5.00, and 10.00g/kg. The positive control compound, cyclophosphamide, was administered intraperitoneally (i.p.) at the dose level of 0.04g/kg. After 35 days, the mice were sacrificed. Both testes were removed, made into slides, and stained following the standard procedure. Under microscope through oil lens, 5000 sperms were examined. The number of abnormal sperms was obtained. The abnormality rate was expressed as abnormal sperms per 1000 sperms examined. The result are shown in table 5.3

5.4.5 Result

Table 5.3 Result of the mouse abnormal sperms

Dose (g/kg)	No. of Animals	No of sperms examined	No. of abnormal sperms	Abnormality rate (0/00)
0	5	5000	98	19.60
10.00	5	5000	98	19.60
5.00	5	5000	94	18.80
2.50	5	5000	92	18.40
Cyclophosphamide (0.04)	5	5000	364	72.80**

** Following the Wilcoson Serial Examination Method, the placebo control group and the dose groups were compared to the cyclophosphamide control group, $P<0.01$

5.4.6 Conclusion

- There was no significant difference between the treatment groups and the placebo control group. The most common abnormality of the sperms in the treatment and placebo control group was unfixed-shape. The next most common abnormality was a swelling head. No other abnormalities were observed. In the cyclophosphamide positive control group, the most common abnormality of the sperms was unfixed-shape. Occasionally, no-hook-shape, banana-shape and curly-tail-shape were observed.

- The Ganoderma Spore Powder at the highest dose of 10g/kg did not induce abnormalities in mouse sperms.

5.5 Ames Test

5.5.1 Testing Institute

- Food Safety and Inspection Laboratory, Institute of Health, Guang Dong Province, China.

5.5.2 Testing Date

- 16th November, 1999

5.5.3 Material

- Testing compound: 120g *Ganoderma lucidum* Spore Powder was used, sieved and mixed with 300ml of distilled water.

5.5.4 Methods

- The bacterial strains (TA97, TA98, TA100 and TA102) were provided by the Beijing Food Inspection Office, Department of Health, China. The bacterial strains were examined for certain characteristic and S9 reactivity and were found suitable for the Ames test. The Ames test in the presence and absence of S9 extract was performed according to standard procedure. Duplicate experiments were performed independently. Three plates were used for each dose level. The result are shown in Table 5.4

5.5.5 Result

Table 5.4 Result of the Ames test with *Ganoderma lucidum* Spore Powder

Dose	**TA97**		**TA98**		**TA100**		**TA102**	
(μg/plate)	*+S9*	*-S9*	*+S9*	*-S9*	*+S9*	*-S9*	*+S9*	*-S9*
5000	149	135	33	32	180	167	311	296
500	154	141	37	34	148	152	311	295
50	161	152	47	36	175	164	305	288
5	149	153	35	30	167	159	299	267
0.5	164	159	38	35	153	146	305	288
Natural mutation	145	142	40	39	167	154	311	297
Negative control	150	144	39	38	166	160	306	298
Dexon		>1500		>1400				
NaN_3						>1500		
MMC								>1500
2-AF	>1500		>1000		>1500			

5.5.6 Conclusion

- The number of mutated colonies in the treatment groups were comparable to those of the naturally occurred mutations for the four bacterial strains tested, regardless of the doses and the presence or absence of the S9 mixture. In contrast, the positive control group (2-AF, Dexon, NaN_3 and MMC) showed markedly higher number of mutated colonies than the naturally occurred mutation. It is concluded that *Ganoderma lucidum* Spore Powder did not induce mutation in the Ames test.

Pharmacological & Clinical Studies in Literature

6. *Pharmacology and Clinical Studies of Ganoderma lucidum Reported in the Literature*

Over one hundred oxygenated triterpenens have been isolated from *Ganoderma lucidum*. Of these, ganoderic acid and β lucidumol B, ganodermanonodiol, ganodermanontriol and ganolucidic acid A showed activity to ingibit human immunodeficiency virus (HIV) protease in a recent report (Min BS *et al.*, 1998). A new protein, LZ-8 has been isolated from *Ganoderma lucidum* and shown to be a member of the immunoglobulin superfamily (Kino K *et al*, 1991, van der Hem LG *et al.*, 1995). Treatment with LZ-8 prevented BSA-induced systemic anaphylaxis in CFW mice (Kino K *et al*, 1989) and the development of autoimmune type I diabetes in NOD mice (Kino K *et al.*, 1990). These result demonstrated an immunosuppressive action of *Ganoderma lucidum* in vivo. Ganoderma Lucidum is very rich in polysaccharides. It has been found that crude or partially purified polysaccharides of *Ganoderma lucidum* significantly inhibited the growth of locally implanted A180 sarcoma and reduced tumor metastasis in mice (Miyazaki T *et al.*, 1981, Maruyama H *et al.*, 1989) Wang (Wang *et al.*, 1997) discovered that the anti-tumor effect of *Ganoderma lucidum* polysaccharides is mediated by cytokines released from activated macrophages and T lymphocytes treated with *Ganoderma lucidum*. Another study (Lieu CW *et al.*, 1992) was consistent with this conclusion, demonstrating that the polysaccharide fraction of *Ganoderma lucidum* (PS-G) induced differentiation of human leukemic cell line U937. However, the differentiation was induced by the conditioned medium from PS-G stimulated human blood mononuclear cell. PS-G itself did not have any effect on the target cells. Yet another pharmacological effect of *Ganoderma lucidum* is its anti-hypertensive action and an inhibitory effect on angiotensin coverting enzyme (ACE)(Lee SY *et al.*, 1990, Morigiwa A *et al.*, 1986). Extract of *Ganoderma lucidum* was shown to decrease systolic and diastolic blood pressure in rabbits, probably due to its central inhibitory effect on the sympathetic nerves. Furthermore, the water-soluble fraction of *Ganoderma lucidum* was shown to suppress platelet aggregation of bovine blood in vitro (Shimizu A *et al.*, 1985).

In the literature, mice were able to tolerate intraperitoneal injection of water- or

alcohol-soluble extraction of *Ganoderma lucidum* spore up to 40g/kg body weight (Liu G *et al.*, 1979)

In a clinical study, 10 patients (8 males and 2 females) with atrophic myotonia were given a water-soluble preparation of *Ganoderma lucidum* spores by intramuscular injection (Fu HD *et al.*, 1982). The patients received an initial dose of 400-800mg/day for a period of time for a total dosage of 38.4 – 180g. The patients were followed up from 8 months to 6.8 years. No short-term and long-term adverse reactions were observed.

A clinical evaluation of the anti-hypertensive effect of lyophilize *Ganoderma lucidum* extract was reported by Kanmatsuse (Kanmatsuse *et al.*, 1985). 53 patients with either essential hypertension, mild hypertension, or normal blood pressure were given *Ganoderma lucidum* extract as oral tablet. Each patient took 6 tablet containing 240mg of the extract every day for a period of 6 months. The result showed that *Ganoderma lucidum* had blood-pressure lowering effect on patients with essential hypertension, and did not have any side effects on patients with essential or border line hypertension during 6 months of oral intake.

Conclusion

7. *Conclusion*

Based upon the toxicity study result, we concluded that *Ganoderma lucidum* Spore Powder is considered safe to use at the recommended maximum oral daily dose of 4.8g (300mg / capsule, 4 capsule to be taken 3-4 times daily)

Reference

8. *Reference*

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Appendix A
Testing Report in China
(Chinese)

CNACL
No.0623

广东省疾病预防控制中心
（广东省卫生检验中心）

检　验　报　告

收样日期：2002 年 03 月 14 日
受理编号：01GA1042　　检验日期：2002 年 04 月 08 日

样品名称：学者牌纯灵芝孢子粉胶囊	检验类别：卫生许可
生产单位：广州绿色食品工程公司	样品批号：20010910 20010925 20011012
送检单位：广州绿色食品工程公司	样品数量：250g/批
送检单位地址：中山大学测试大楼五楼	样品性状：胶囊
实验室名称：微生物检验所	包装情况：0.3g/粒×40 粒/瓶,塑料瓶装
实验室地址：广州市新港西路 176 号	

一、检验方法及说明：

按照中华人民共和国国家标准 GB4789.2、3、4、5、10、11、15-94 食品卫生检验方法微生物学部分进行检验。

二、检验项目和检验结果：

检测项目	检测结果		
批号：	20010910	20010925	20011012
菌落总数（cfu/g）	<10	<10	10
大肠菌群（MPN/100g）	<30	<30	<30
霉菌计数（cfu/g）	10	10	<10
酵母计数（cfu/g）	<10	<10	<10
沙门氏菌	未检出	未检出	未检出
志贺氏菌	未检出	未检出	未检出
金黄色葡萄球菌	未检出	未检出	未检出
溶血性链球菌	未检出	未检出	未检出

（以下空白）

检验报告专用章

检验　2002 年 4 月 22 日　　校核　2002 年 4 月 22 日　　复核　2002 年 4 月 22 日　　签发　2002 年 4 月 22 日

中山医科大学保健食品检测中心

理化检验报告

样品受理编号：SHU20011212　　报告编号：HBG200201007　　报告日期：2002年1月29日

收样日期：2001.12.12.　　检验日期：2002.1.8~28.

检品名称：学者牌纯灵芝孢子粉胶囊　　样品性状：

送检单位：广州绿色食品工程公司

检验项目：多糖、不饱和脂肪酸、水分等

检验依据：

检验结果：

项目 \ 批号		010910	010925	011012	
水分	(g/100g)	2.45	2.40	2.41	BG5009.3-85
铅	(mg/kg)	0.34	0.31	0.31	GB/T5009.12-1996
砷	(mg/kg)	<0.1	<0.1	<0.1	GB/T5009.11-1996
汞	(mg/kg)	0.020	0.019	0.022	GB/T5009.17-1996
六六六	(mg/kg)	<0.01	<0.01	<0.01	GB/T5009.19-1996
滴滴涕	(mg/kg)	<0.01	<0.01	<0.01	GB/T5009.19-1996
多糖	(g/100g)	3.12	3.23	3.07	硫酸苯酚比色法
不饱和脂肪酸	(%)	25.05	25.54	28.04	参照企业标准方法

(以 下 空 白)

中山医科大学保健食品检测中心

检验人：　　核对人：　　负责人：

说明：1. 本报告各页及其复印件未加盖本单位印章无效。

2. 本报告结果只对来样负责，不得用作商业宣传。

3. 若对本报告结果有异议，请在报告发出之日起30天内来本单位复测，逾期不予受理。

地址：广州市中山二路74号　邮编：510089

电话：（020）87330617　电传：020-87330446　E-mail: gwyy@gzsums.edu.cn

Appendix B
Toxicity Testing Report in China (Chinese)

广东省卫生防疫站

毒理检验报告

收样日期：1999.09.02.

(99) 粤食卫毒检字第 054 号

报告日期: 1999.12.15

样品名称	学者牌纯灵芝孢子粉胶囊（全破壁）	商　标	学者牌
生产厂家	广州绿色食品工程公司	样品来源	抽　检
样品状态及包装	棕色粉末状，胶囊装	采或送样人	陈卫东
编号或批号	990805	样品数量	3 公斤
检测项目	30 天喂养试验		
检测依据	食品安全性毒理学评价程序和方法 GB15193　1—94		

检测结果:

本试验用掺入（100g 粉饲料计）2.5、5、10g 不同量的学者牌纯灵芝孢子粉的饲料喂养生长期 SD 种雌雄大鼠，共 30 天，终期结果如下：

1、大鼠一般生理体征、行为、大小便、皮毛等均无异常。

2、各剂量组大鼠的体重增长、脏体比值和食物利用率指标与空白对照组比，均无显著差异，鼠生长发育良好。

3、血象常规检查结果基本正常。

4、各项血液生化指标值均正常。

5、脏器病理切片观察未发现异常。

(以下空白)

(公章)

检验者 何洁莹　复核者 [illegible]　审核者 黄[illegible]　签发人 [illegible]

注: 本单一式二联, 第一联由监督机构存档, 第二联送被监督单位。

学者牌纯灵芝孢子粉胶囊（全破壁）毒理学检验报告

（一）材料：

1.棕色粉末，胶囊装.推荐日服量为:4 次/天×4 粒/次×0.38g/粒，按成人体重 60 公斤计，推荐量为 0.08g/kg. b.w.。

2.动物:广东省医用动物场提供的 SD 种健康大鼠。

（二）试验方法:

选用纯种 SD 健康大鼠，广东省医用动物场提供，体重 80-88 克，共 96 只，随机分 4 组， 每组 24 只，雌雄各半，各组体重均差不超过± 5 g，投药前观察 1 周，观察各组动物活动、进食、外观体征等是否有异常。

1. 剂量：试验时将样品掺入大鼠饲料粉内制成含不同量灵芝孢子粉的饲料条，供试验用。剂量组如下：

空白对照组：不含样品的饲料

低剂量组： 100 克的饲料粉掺入 2.5 克样粉

中剂量组： 100 克的饲料粉掺入 5.0 克样粉

高剂量组： 100 克的饲料粉掺入 10 克样粉

2. 试验方法:

(1).每天分别给足够饲料自由摄影食和饮水,连续给样 30 天，每周称体重及计算饲料消耗量，并观察动物的生理指标。

(2).试验结束时作血常规检查，项目为红细胞计数、血色素、白细胞计数、分类及血小板数，用日本 R-1000SYSME 血球计数仪测定。血液生化指标查血糖、白蛋白、甘油三酯、总胆固醇、肌酐、谷丙转氨酶和尿素氮。用法国 ALIZE 全自动生化分析仪测定。

（3）.取肝、肾、脾、心及睾丸称重后用甲醛固定，按常规切片，染色，观察病理变化。

3. 结果与分析：

(1).各剂量组大鼠生长发育情况良好、大鼠一般生理体征、行为、大小便、皮毛等均无异常。各组各周期大鼠的体重增值、摄食饲料量、食物利用率与对照组比较差异无显著性 （P>0.05）。（见表 1 、表 2）

(2).终期血象测定，雄性白细胞升高，F=5.14，低、中剂量组与空白组比较有显著性差异（P<0.05），但波动在 SD 大鼠 4—15.4×10^9 正常范围内，其余各项指标与对照组比较无差异（P>0.05），见表（3）

(3).终期生化指标：雌性中、高剂量组血糖升高，F=3.93，与对照组比较有显著性差异（P<0.05），但波动在 SD 大鼠 4—9.8mmol/L 正常范围内。雄性低、中剂量组尿素氮降低，F=4.49，与对照组比较有显著性差异（P<0.05），但波动在 SD 大鼠 5.2—8.6μmol/L，范围内其它各项指标与对照组比较无显著性差异（P>0.05）。（见表 4）

（4）各脏体系数试验组与对照组比较无显著性差异。（见表 5）.病理学观察：各剂量组脏器病理学未发现异常。

（表 1）　　大鼠体重增长情况　　每组 n=12　　X±SD

性别	组别	始重	第一周	第二周	第三周	第四周
雄鼠	对照	87.10±9.40	120.20±11.00	152.10±12.90	192.00±13.40	242.30±17.60
	低	88.00±6.70	125.10±9.30	145.00±9.90	190.60±11.50	242.00±18.20
	中	85.70±8.60	125.80±15.20	148.40±12.10	192.90±12.20	235.50±24.00
	高	86.50±8.90	123.00±13.70	154.70±17.00	202.20±18.30	251.60±25.80
雌鼠	对照	82.40±7.50	109.20±8.00	148.80±8.20	165.10±14.00	202.60±16.10
	低	80.50±7.30	117.70±9.20	144.30±9.90	174.20±12.00	206.20±11.50
	中	80.50±5.90	112.40±15.20	141.20±9.90	171.50±13.10	199.60±17.20
	高	81.70±6.60	115.30±13.00	144.10±14.70	171.60±16.20	206.80±24.90

（表 2）　大鼠体重增长、摄取饲料量、食物利用率　每组 n=12　X±SD

性别	组别	始重（克）	终重（克）	增重（克）	饲料摄入量 (g/只)	食物利用率（%）
雄鼠	对照	87.10±9.40	242.30±17.60	155.20±20.10	596.40	26.02
	低	88.00±6.70	242.00±18.20	154.00±15.50	656.70	23.45
	中	85.70±8.60	235.50±24.00	149.80±24.30	625.60	23.95
	高	86.50±8.90	251.60±25.80	165.10±19.50	640.90	25.76
雌鼠	对照	82.40±7.50	201.60±16.10	119.20±19.50	552.80	21.56
	低	80.50±7.30	206.20±11.50	125.70±11.80	574.80	21.87
	中	80.50±5.90	199.60±17.20	119.10±14.70	569.20	20.92
	高	81.70±6.60	206.80±24.90	125.10±25.50	565.70	22.11

（表 3）　　血常规指标　　每组 n=12　　X±SD

性别	组别	红细胞 (×10 ^{12}个/L)	血红旦白 (g/L)	血小板 (×10 ^{9}/L)	白细胞 (×10 ^{9}/L)	淋巴细胞 (%)	中间型细胞 (%)	中性细胞 (%)
雄鼠	对照	6.84±0.36	124.20±10.10	1063.20±167.40	7.17±1.23	70.42 ±5.38	5.50±3.15	24.08±2.39
	低	6.57±0.51	122.20±13.80	931.80±90.90	10.85±3.53*	70.92±3.85	4.25±1.71	25.00±2.08
	中	6.51±0.41	122.50±14.10	981.70±190.30	9.40±1.86*	70.58±3.92	4.83±1.70	24.58±2.68
	高	6.71±0.38	123.20±10.60	1169.80±254.20	8.12±2.00	71.33±2.50	4.75±1.36	23.92±1.56
雌鼠	对照	6.74±0.66	132.00±10.10	1155.70±196.30	9.63±3.39	71.25±4.69	4.33±2.00	24.42±2.78
	低	6.32±0.62	126.10±2.90	1202.50±256.60	10.68±2.89	73.50±2.75	3.92±1.56	22.75±1.42
	中	6.43±0.91	133.20±9.20	1241.80±199.60	8.73±1.79	70.33±4.36	4.83±1.80	23.17±3.74
	高	6.33±0.50	127.80±7.70	1440.20±377.50	10.42±1.19	72.17±4.22	4.13±1.92	23.75±2.42

*与对照组比 P<0.05

（表 4）　　生化指标　　每组 n=12　　X±SD

性别	组别	血 糖 (mmol/l)	甘油三脂 (mmol/l)	总胆固醇 (mmol/l)	尿素氮 (mmol/l)	谷丙转氨酶 (IU/L)	血清白旦白 (g/L)	肌酐 (μ mol/l)
雄鼠	对照	2.65±0.67	1.41±0.37	1.78±0.23	10.29±1.61	51.00±7.60	38.09±1.42	66.76±4.91
	低	2.65±0.67	1.67±0.44	1.98±0.30	8.64±1.32*	55.80±10.50	40.73±1.72	65.57±6.52
	中	2.75±0.41	1.63±0.42	1.90±0.41	8.40±1.58*	55.80±11.70	41.42±1.39	66.57±5.52
	高	3.08±0.48	1.36±0.39	1.73±0.36	9.44±2.07	59.10±10.90	40.91±0.91	67.56±4.91
雌鼠	对照	3.71±0.54	0.99±0.16	1.83±0.29	8.88±1.50	48.00±8.30	40.72±0.96	70.60±6.26
	低	3.75±0.59	1.10±0.25	1.94±0.28	9.24±0.95	53.80±11.90	40.28±1.44	70.33±4.23
	中	4.24±0.37*	0.92±0.20	1.78±0.22	9.99±1.42	54.10±6.90	41.69±1.38	73.98±6.14
	高	4.27±0.55*	1.02±0.23	1.99±0.39	8.95±2.07	51.60±13.20	41.86±2.56	74.84±7.81

*与对照组比 P<0.05

(表十)　　脏体系数比　　每组 n=12　　X±SD

性别	组别	心	肝	脾	肾	睾丸
雄鼠	对照	0.31±0.03	2.67±0.18	0.24±0.03	0.63±0.05	0.86±0.09
	低	0.31±0.03	2.60±0.18	0.26±0.05	0.64±0.04	0.82±0.12
	中	0.30±0.03	2.60±0.45	0.24±0.05	0.65±0.07	0.87±0.14
	高	0.31±0.03	2.65±0.17	0.21±0.02	0.63±0.05	0.86±0.08
雌鼠	对照	0.32±0.02	2.44±0.23	0.26±0.05	0.63±0.10	
	低	0.31±0.03	2.47±0.72	0.27±0.03	0.64±0.06	
	中	0.33±0.04	2.24±0.78	0.25±0.78	0.67±0.08	
	高	0.33±0.03	2.45±0.34	0.25±0.05	0.64±0.07	

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中华人民共和国卫生监督文书

卫生检验结果报告单

(1999) 粤卫防食毒检字第 187 号

收样日期：1999.9.2　　报告日期：1999.11.16

样品名称	学者牌纯灵芝孢子粉胶囊（全破壁）	商　标	学者牌
送样单位	广州绿色食品工程公司	样品来源	送检
样品状态及包装	胶囊.玻璃瓶装	采或送样人	黄晓霓
编号或批号	990805	样品数量	1000 克
检测项目	小鼠 LD_{50}、微核、精子畸形及 Ames 试验		
检测依据	食品安全性毒理学评价程序和方法 GB15193 1-94		

检测结果：

1. LD_{50}：

未观察到动物有任何不良反应，获得雌雄小鼠经口 LD_{50}＞21.5g.kg B.W。样品学者牌纯灵芝孢子粉胶囊（全破壁）属无毒级物质。

2. 微核试验

0.62-10g/kg b.w 学者牌纯灵芝孢子粉胶囊（全破壁）的微核率与空白对照组比较，均无显著性差异，试验结果阴性，对体细胞无诱变作用。

3. 精子畸形试验

2.5-10g/kg b.w 学者牌纯灵芝孢子粉胶囊（全破壁）的精子畸形率与空白对照比较，差异无显著性，试验结果阴性，未发现对生殖细胞有诱变畸形作用。

4. Ames 试验

0.5-5000ug/皿学者牌纯灵芝孢子粉胶囊（全破壁）无论加或不加 S9 混合物，试验结果回变菌落数均未超过自然回变菌落数的 2 倍，未发现学者牌纯灵芝孢子粉胶囊（全破壁）有直接或间接的致突变作用，结果阴性。

检验者：　　复核者：　　（公章）签发人：

注：本单一式二联，第一联由监督机构存档，第二联送被监督单位。

学者牌纯灵芝孢子粉胶囊（全破壁）毒理学检验报告

(一)材料：

1.试验材料：样品为棕色粉末。过100目筛后取120g样加300ml蒸馏水于7000转/分搅拌机拌15分钟，装瓶，灭菌消毒。获得1ml膏液相当于0.4g样，采用2次/日直接灌胃。

2.动物:广东省医用动物场提供的NIH种健康小白鼠，体重18-22g。

(二)方法与结果:

1.小鼠急性毒性LD_{50}试验:

NIH种小白鼠40只，体重18-22g，雌.雄各半，采用Horn's法，随机将鼠分为4个剂量组，一次空腹灌胃，观察一周，结果见表(一)。

表(一)　　急性毒性试验结果

剂量 (g/kg)	动物数(只) 雌	雄	动物死亡数(只) 雌	雄
21.50	5	5	0	0
10.00	5	5	0	0
4.64	5	5	0	0
2.15	5	5	0	0

结果:试验小鼠活动、饮食正常，无一死亡，获得雌雄小鼠经口LD_{50}>21.5 g/kg B.W。该样品属无毒级物质。为推荐量（0.08g/kgB.W）的268.75倍。

2.小鼠骨髓微核试验:

NIH种小鼠70只，体重20-23g,分别将鼠分为7组,按"食品安全性毒理学评价程序"方法试验,分两次灌胃，于第二次灌胃6小时后,处死小鼠取两侧股骨材料制片、染色、镜检，求出各组微核率，按泊松分布方法统计。结果见表(二)。

结果：环磷酰胺阳性对照组与空白对照组比较，有极显著性差异；样品各剂量组的微核率与空白对照组比较接近，均无显著性差异，试验结果为阴性。

（表二）　小鼠骨髓微核试验结果

剂量 (g/kg)	动物数(只) 雌	雄	受检嗜多染红细胞数(个) 雌	雄	微核数(个) 雌	雄	微核率(‰) 雌	雄
0	5	5	5000	5000	7	7	1.4	1.4
10.00	5	5	5000	5000	8	7	1.6	1.4
5.00	5	5	5000	5000	6	8	1.2	1.6
2.50	5	5	5000	5000	6	7	1.2	1.4
1.25	5	5	5000	5000	7	7	1.4	1.4
0.62	5	5	5000	5000	6	6	1.2	1.2
环磷酰胺 (0.06)	5	5	5000	5000	123	126	24.6**	25.2**

**与空白对照组比较 $p<0.01$

3. 精子畸变试验

NIH 种小鼠 25 只，体重 18-22 克，随机分为 5 组，连续灌胃 5 天(环磷酰胺阳性组作腹腔注射)，经 35 天后处死动物取双侧副睾按常规制片、染色、油镜下每组检查完整精子 5000 条，求出精子畸形率，按 Wilcoson 方法统计。结果见表(三)。

(表三) 学者牌纯灵芝孢子粉胶囊（全破壁）小鼠精子畸形分析

剂 量 (g/kg)	动物数 (只)	受检精子数 (条)	畸形精子数 (条)	畸形率 (‰)
0	5	5000	98	19.60
10.00	5	5000	98	19.60
5.00	5	5000	94	18.80
2.50	5	5000	92	18.40
环磷酰胺 (0.04)	5	5000	364	72.80 **

**表示与空白对照组比较 P<0.01.

结果:环磷酰胺组与空白对照组比较有极显著性差异；学者牌纯灵芝孢子粉胶囊（全破壁）各剂量组的精子畸形率与空白对照组接近，在剂量达 10.00g/kg B.W 均未发现对生殖细胞有诱变畸形作用。

4. Ames 试验

试验菌株(TA97、TA98、TA100、TA102)由北京卫生部食检所提供，对菌株部分性状和 S9 活性进行鉴定，符合要求，采用平皿掺入法，进行两次独立试验，每组做三个皿，结果见表(四)。

结果:本样品各剂量组中无论加或不加 S9 混合物，试验结果回变菌落均未超过自然回变菌落数的 2 倍，未发现该样品有直接或间接的致突变作用。

(表四) 学者牌纯灵芝孢子粉胶囊（全破壁）平皿掺入法检测结果

剂 量	TA97		TA98		TA100		TA102	
ug/皿	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
5000	149	135	33	32	180	167	311	296
500	154	141	37	34	148	152	311	295
50	161	152	47	36	175	164	305	288
5	149	153	35	30	167	159	299	267
0.5	164	159	38	35	153	146	305	288
自然回变	145	142	40	39	167	154	311	297
溶剂对照	150	144	39	38	166	160	306	298
阿的平		>1500		>1400				
叠氮钠						>1500		
丝裂霉素								>1500
2-氨基芴	>1500		>1600		>1500			
顺铂							>2500	